Am. J. Trop. Med. Hyg., 82(6), 2010, pp. 1185–1193
doi:10.4269/ajtmh.2010.09-0324

Temporal Connections between *Culex tarsalis* Abundance and Transmission of Western Equine Encephalomyelitis Virus in California

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Abstract. Definition of targets for vector control requires an understanding of the relationship between vector abundance and the intensity of arbovirus transmission. Using an extensive surveillance dataset with observations from sentinel chicken flocks and mosquito traps paired in time and space, hierarchical autoregressive logistic regression models were developed to predict the probability of seroconversion in chickens for western equine encephalomyelitis virus (WEEV) based on the relative abundance of the principal vector, *Culex tarsalis.* After adjustments for confounders, the abundance of *Cx. tarsalis* 29–42 d before the date of chicken sampling was credibly associated with the risk of WEEV transmission in both the Central and Coachella Valleys, and a doubling of relative *Cx. tarsalis* abundance was associated with a 58% increase in the odds of seroconversion. The critical time windows identified in our study highlight the need for surveillance of vector populations and forecasting models to guide proactive vector control measures before the detection of transmission to sentinel chickens.

INTRODUCTION

Arboviral control strategies typically are based on the tenet that transmission risk to humans is positively associated with vector abundance and that suppressing vector abundance will reduce the intensity of virus transmission.[1,2] The equation for vectorial capacity—developed as a model for the entomological components of malaria transmission[3] and widely regarded as a useful expression for other vector-borne pathogens[4–6]—suggests a positive, linear relationship between the density of vectors and the force of pathogen transmission. Although the basic reproduction numbers of vector-borne pathogens are more sensitive to changes in other parameters, such as vector survival, the density of vectors varies over a broader range than most other parameters and remains the most directly measurable component of vectorial capacity.

Previous studies have suggested that the relationship between vector abundance and arbovirus transmission risk might not be linear, instead having thresholds below which human cases and transmission cease.[7,8] A study of mosquito and arboviral surveillance records from 1953–1973[9] found that seasonal indices of mosquito abundance were positively correlated with human case incidence and sentinel chicken seroconversion rates, except at the highest mosquito abundance levels, which had little arbovirus activity. Despite a long history of arboviral surveillance in California, the prerequisites for epizootic transmission are not well understood. One reason is that mosquito abundance and arboviral transmission are focal, defined by relatively small spatial and temporal dimensions, and previous studies have evaluated surveillance measures aggregated over broad areas and time periods, which might mask small-scale patterns.[10,11] Furthermore, these studies generally measured associations using simple correlations or regression, but the necessary conditions for viral amplification are likely to be multivariate, relying on the convergence of optimal climatic conditions, vector abundance, and susceptible avian hosts in space and time.

Here, we considered western equine encephalomyelitis virus (*Togaviridae, Alphavirus,* WEEV), which is an arbovirus maintained by transmission between the principal mosquito vector, *Culex tarsalis,* and several avian amplifying hosts. The WEEV causes encephalitis in humans and horses and has been a focus of vector control programs in California since the discovery that it was transmitted primarily by *Cx. tarsalis* mosquitoes[12,13] and caused neuroinvasive illness.[14–16] In recent decades, encephalitis cases in humans and horses caused by WEEV infections have almost disappeared from western North America because of increased equine vaccination,[17] improved irrigation practices that reduce standing water,[18] expanded vector control and surveillance, and human behavioral changes that have reduced exposure to mosquitoes.[19] However, enzootic WEEV activity is still detected sporadically in many areas of California,[20,21] with activity during some years involving extensive areas.[11,22] Genetic similarities in viral isolates among years have suggested that WEEV may persist in enzootic foci,[23] and a recent comparison of viral isolates from 1953–2005 showed that the host competence of sparrows and the vector competence of *Cx. tarsalis* were not associated with the year of viral isolation, suggesting that viral attenuation could not explain the decline in human cases despite continued enzootic transmission.[24]

In the current study, we used Bayesian hierarchical logistic regression models fitted to an extensive WEEV and *Cx. tarsalis* surveillance dataset to test the hypothesis that increased *Cx. tarsalis* female abundance favors WEEV transmission to sentinel chickens after adjustment for confounders, such as temperature and among-flock variation in baseline seroconversion risks. Unlike earlier studies,[7,9] time within each season was explicitly incorporated into the analysis in the form of temperature and abundance variables within time intervals before each chicken sampling date and autoregressive terms connecting each half-month to the next within a season. This resulted in a more comprehensive and interpretable characterization of the preconditions for epizootic WEEV transmission.

METHODS

Study area and time period. Our study included 41 sentinel chicken flocks in 14 vector control agencies located in the Central and Coachella Valleys of California (Figure 1).

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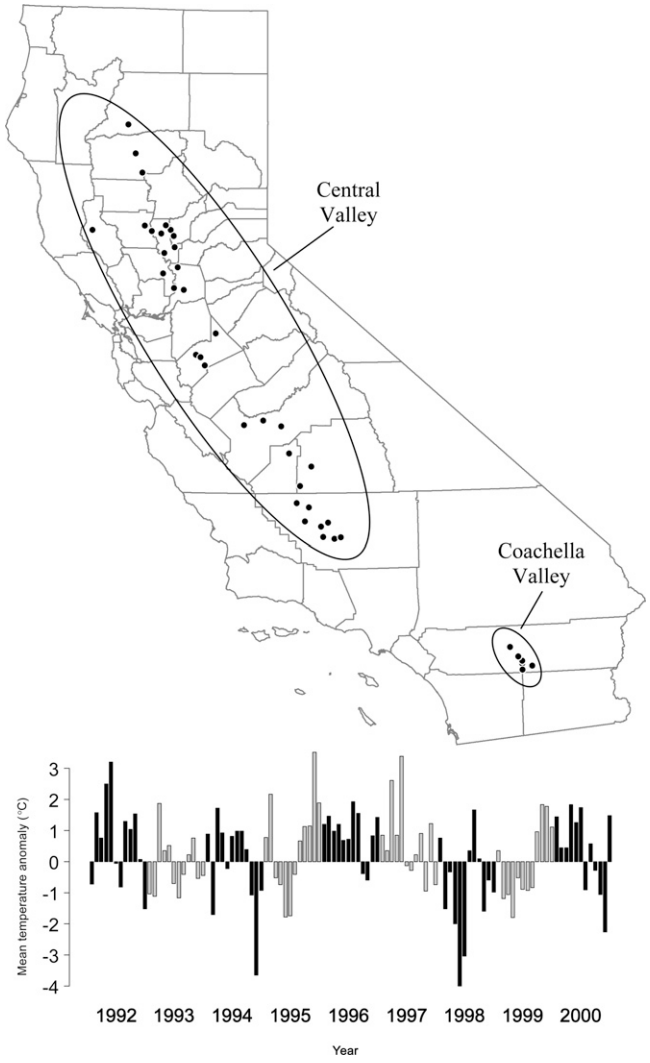

FIGURE 1. Map of California showing the chicken flock sites included in this study and boundaries for counties. The locations of the Central and Coachella Valleys are indicated. The lower graph shows mean monthly temperature anomalies as an indication of year-to-year variation during the 1992–2000 study periods.

These areas were selected for study because of their history of sporadic WEEV activity.[20,21] The years 1992–2000 were chosen because 1) they had varying degrees of WEEV activity, including extensive enzootic activity in the Sacramento Valley during 1993[11]; 2) they covered time periods for which paired mosquito trapping records were available; and 3) flocks were relatively consistent statewide in terms of size (10 chickens/flock) and bleeding schedule (biweekly, i.e., every 2 weeks).

Temperature. Daily maximum and minimum temperatures were acquired from the National Aeronautics and Space Administration's Terrestrial Observation and Prediction System (TOPS; http://ecocast.arc.nasa.gov).[25] TOPS uses weather and ecosystem models to combine ground-based and remotely sensed inputs to generate multiple measures of environmental conditions. For this study, the TOPS Surface Observation and Gridding System (SOGS)[26] was used to ingest daily observations of maximum and minimum temperature from ~700 ground-based meteorological stations throughout California. From these station observations, daily temperature surfaces were interpolated to spatially continuous grids[27] at a 1 km^2 resolution based on the spatial convolution of a truncated Gaussian filter. The interpolation algorithm also accounted for elevation differences using an empirical relationship calculated for each daily grid by weighted least squares regression.[27] Mean absolute errors for the SOGS 8-km grids for the United States are 1.9°C for maximum temperature surfaces and 2.0°C for minimum temperature surfaces,[26] but errors for our study area typically are smaller because of the low topographic relief and dense network of ground stations, which are ideal for interpolation of meteorological surfaces.

Temperatures were extracted from the gridded meteorological surfaces for individual flock sites, and daily temperatures were aggregated for each of several time periods as the mean daily minimum (T_{MIN}), mean daily maximum (T_{MAX}), and total degree-days greater than the estimated WEEV transmission threshold of 10.9°C (DD_{WEEV}).[28] Degree-days were calculated using the single-triangle method.[29] The time periods for aggregation were defined in two ways: 1) as monthly variables from Jan–May to measure possible associations with virus overwintering and spring amplification, and 2) as lagged variables for 14-d windows up to 8 weeks before the bleed date, i.e., 8–21, 15–28, 22–35, 29–42, 36–49, and 43–56 d. The 14-d lagged intervals were chosen to measure more immediate effects on viral replication rates in mosquito vectors and vector feeding frequency at a temporal scale equal to that of the biweekly sentinel chicken sampling. The week immediately preceding each chicken sampling date was excluded from lag periods for temperatures and *Cx. tarsalis* abundance because expression of detectable antibodies to WEEV in chickens does not occur until ≥ 8 days after an infectious mosquito bite.[30] Therefore, transmission risk from exposures just before the sampling date would not become evident until the following sampling date.

***Cx. tarsalis* abundance.** The abundance of *Cx. tarsalis* was estimated by New Jersey-style light traps (NJLT)[31] located at sites that were paired with a nearby chicken flock. Paper or electronic mosquito collection records were obtained from historical archives maintained by the individual vector control agencies. Records received in paper format were entered into a PostgreSQL database before analysis. Traps typically were operated by local districts continuously throughout the Apr–Oct surveillance season and monitored at 7-d intervals. Prior to analysis, total numbers of *Cx. tarsalis* females and trap-nights were aggregated by the month or lag period within which the ending date of the trapping period fell. Trap counts were aggregated in the same way as temperatures (i.e., monthly from Jan–May and staggered 14-d intervals preceding each chicken sampling date) using structured query language (SQL).

WEEV transmission. Sentinel chicken flocks were maintained by local vector control agencies (Table 1), and a blood sample was taken by agency personnel biweekly from each chicken by pricking the comb and collecting a small amount blood on a filter paper strip. The filter paper strips were shipped to the Viral and Rickettsial Diseases Laboratory of the California Department of Public Health (CDPH). Initial testing of chicken sera for WEEV IgG was done by enzyme immunoassay (EIA), which has been shown to be approximately equal in sensitivity and specificity to the gold-standard plaque-reduction neutralization test for WEEV.[30] Data on sentinel chicken seroconversions and numbers tested for each flock were assembled from annual reports published in

TABLE 1

Summary showing the total number of WEEV seroconversions/total chicken-bleeds per flock per year, 1992–2000

Agency	Nearest city	Flock code	Year									TOTALS
			1992	1993	1994	1995	1996	1997	1998	1999	2000	
Colusa MAD	Colusa	CLSA0001	0/87	6/60	3/80	1/71	0/80	2/82	0/70	0/83	0/100	**12/713**
Coachella Valley MVCD	Mecca	COAV0035	1/98	0/100	0/115	5/81	0/80	2/80	3/85	0/100	0/95	**11/834**
Coachella Valley MVCD	Desert Beach	COAV0060	8/88	0/101	0/104	4/77	0/76	0/96	6/82	0/100	0/99	**18/823**
Coachella Valley MVCD	Valerie	COAV0131	4/89	0/92	0/105	3/76	0/84	0/99	2/88	0/99	0/99	**9/831**
Coachella Valley MVCD	Mecca	COAV0200						0/91	0/81	0/97	0/100	**0/369**
Coachella Valley MVCD	Thermal	COAV0201				0/80	0/80	1/94	1/88	0/97	0/99	**2/538**
Coachella Valley MVCD	Indio	COAV0202	0/91	0/100	0/100	0/79	0/83	0/90	0/81	0/96	0/91	**0/811**
East Side MAD	Oakdale	EAST1933	0/99	10/100	0/90	0/90	0/91	0/100	0/78	0/101	0/112	**10/861**
Fresno MVCD	Easton	FRNO0001	0/62	0/100	0/68	0/80	0/84	0/80	0/90	0/70	0/80	**0/714**
Fresno MVCD	Kerman	FRNO0002	0/74	0/100	0/60	0/76	2/84	0/80	0/90	0/70	0/80	**2/714**
Fresno Westside MAD	Mendota	FRWS0001	0/97	0/92	0/60	0/80	0/90	0/91	0/54	0/90	0/90	**0/744**
Kern MVCD	Wasco	KERN0100	0/100	0/100	0/80	0/80	7/100	0/90	0/88	0/90	0/90	**7/818**
Kern MVCD	Lost Hills	KERN0101	0/97	0/100	0/77	0/80	8/100	1/90	6/97	0/90	0/90	**15/821**
Kern MVCD	Buttonwillow	KERN0102	0/100	0/100	0/80	0/80	3/100	1/90	0/82	0/90	0/90	**4/812**
Kern MVCD	Old River	KERN0104	0/100	0/100	0/70	0/90	3/90	0/90	2/83	0/90	0/90	**5/803**
Kern MVCD	Pumpkin Center	KERN0105						0/90	3/85	0/90	0/90	**3/355**
Kern MVCD	Bakersfield	KERN0106	0/100	0/100	0/80	0/90	0/100	0/90	0/76	0/90	0/90	**0/816**
Kern MVCD	Arvin	KERN0107	0/99	0/100	0/80	0/90	0/100	0/90	2/90	0/90	0/89	**2/828**
Kern MVCD	Arvin	KERN0108	0/100	0/100	0/80			0/90	0/94	0/89	0/90	**0/643**
Kern MVCD	Bakersfield	KERN0110	0/99	0/100	0/60						0/90	**0/349**
Kings MAD	Hanford	KNGS2001	0/46	0/44	0/90	0/100	0/100	3/90	0/75	0/90	0/100	**3/735**
Lake VCD	Upper Lake	LAKE1000	0/100	6/75	3/76	0/90	4/81	7/66	0/56	0/45	0/99	**20/688**
Sacramento-Yolo MVCD	Woodland	SAYO6002	0/68	7/59	2/56	4/82	1/76	5/55	0/90	0/90	0/90	**19/666**
Sacramento-Yolo MVCD	Elk Grove	SAYO7000	0/100	2/86	0/90							**2/276**
Sacramento-Yolo MVCD	Hood	SAYO7002		6/72	1/75	1/78	8/63	6/58	0/90	0/90	0/82	**22/608**
Sacramento-Yolo MVCD	Natomas	SAYO7004	0/80	2/74	0/90	1/87	2/85	0/80	0/90	0/90	0/90	**5/766**
Shasta MVCD	Anderson	SHAS9704	0/62	0/99	0/88	0/98	0/88	3/68	0/66	0/83	0/88	**3/740**
Sutter-Yuba MVCD	Marysville	SUYA0007	0/100	7/109	2/64	0/100	4/67	3/82	0/80	0/65	0/100	**16/767**
Sutter-Yuba MVCD	Olivehurst	SUYA0010	0/80	8/52	1/87	0/110	1/95	7/66	0/91	0/82	0/90	**17/753**
Sutter-Yuba MVCD	Sutter	SUYA0014	0/80	8/95	0/87	6/82	6/74	9/97	1/93	0/74	0/60	**30/742**
Sutter-Yuba MVCD	Robbins	SUYA0015	0/100	8/109	0/80	0/100	3/86	4/99	0/90	0/74	0/90	**15/828**
Sutter-Yuba MVCD	Meridian	SUYA0020				0/100	0/67	6/68	0/90	0/75	0/90	**6/490**
Sutter-Yuba MVCD	Live Oak	SUYA0030	0/91	6/69	0/88	0/73	5/70	2/89	0/80	0/80	0/90	**13/730**
Sutter-Yuba MVCD	Rio Oso	SUYA0031	0/60	6/80	0/88	3/108	6/66	6/67	0/81	0/80	0/80	**21/710**
Tehama MVCD	Red Bluff	TEHA0001	0/49	7/65	6/67	0/90	8/67	5/62	0/42		0/72	**26/514**
Tehama MVCD	Corning	TEHA0002				0/90	1/96	2/77	0/70		0/72	**3/405**
Tulare MAD	Alpaugh	TLRE0003	0/88	0/56	0/70	0/95	1/82	0/70	0/89	0/99	0/89	**1/738**
Tulare MAD	Tulare	TLRE0008	0/36	1/80	0/70	0/100	0/90	0/66	0/88	1/94	0/72	**2/696**
Turlock MAD	Grayson	TRLK9701			0/88			0/99	0/75	0/99	0/90	**0/451**
Turlock MAD	Patterson	TRLK9702		1/113	0/77		1/95	2/87	0/99	0/99	0/90	**4/660**
Turlock MAD	Crows Landing	TRLK9703	0/92	2/108	0/81	0/117	0/99	9/50	0/99	0/88	0/87	**11/821**
		TOTALS:	**13/2,812**	**93/3,090**	**18/2,901**	**28/3,000**	**74/2,969**	**86/3,199**	**26/3,216**	**1/3,219**	**0/3,575**	**339/27,981**

the *Proceedings and Papers of the Mosquito and Vector Control Association of California* and weekly *Arbovirus Surveillance Bulletins* published by the Vector-borne Disease Section of the CDPH. The WEEV seroconversions were extremely rare during the period from Nov–May, so the chicken datasets used in this study were restricted to sampling intervals falling completely within Jun–Oct.

Statistical analysis. Hierarchical Bayesian logistic regression models were used to relate binomial seroconversion outcomes for each flock and bleed date to relative *Cx. tarsalis* abundance. The model for the number of WEEV-positive chickens, y_{it}, out of n_{it} tested from flock i at time t was as follows:

$$y_{it} \sim \text{Binomial}\ (p_{it}, n_{it}),$$

where the probability of WEEV seroconversion, p_{it}, was related to a series of q fixed predictors, potentially including interactions, by

$$\text{logit}(p_{it}) = \beta_0 + \beta_1 X_{1it} + \ldots + \beta_q X_{qit} + \theta_i + \omega_{it},$$

and random intercepts shared by chickens in the ith flock were defined as

$$\theta_i \sim \text{Normal}(0, \sigma^2),$$

with temporal random terms defined as

$$\omega_{it} \sim \text{Normal}(0, \tau),$$

for t equal to the first half-month of each year, and

$$\omega_{it} \sim \text{Normal}(\gamma^* \omega_{i(t-1)}, \tau),$$

for all other t, where γ is a coefficient to be estimated, connecting each half-month after the first within each year to the one immediately prior.[32,33] Collectively, this structure results in temporal connections for all half-months within a season, with the strength of the connection weakening with greater separation in time, and the temporal connections were assumed to be the same for both valleys. Non-informative priors or hyperpriors were assigned for β [Normal(0, 100)], τ [Gamma(1, 1)], σ [Uniform(0, 10)], and γ [Uniform(−5, 5)].

Models were fitted in WinBUGS version 1.4.3[34] using the R2WinBUGS package in R version 2.6.2.[35] Posterior distributions for all parameters were sampled from each of three chains for 5,000 iterations following a 5,000-iteration burn-in, for a total of 15,000 samples. Each chain was assigned random start values from a Normal(0, 1) distribution for θ and β, a Uniform(0, 1) for σ, and a Uniform(−1, 1) for γ. Convergence was assessed by the Gelman-Rubin statistic.[36]

Variable selection was based on the deviance information criterion (DIC),[37] credibility of parameters, and biological considerations. Regression parameters with 95% credible intervals (CIs) that excluded 0 were considered to be credible. The association between relative *Cx. tarsalis* abundance and WEEV transmission was of primary interest, so adjustments were considered for temperatures based on direct effects on WEEV transmission and as confounders that have direct and indirect effects on both *Cx. tarsalis* abundance and WEEV transmission. Thus, best-fit temperature variables and time intervals were chosen first and included in all subsequent models for selection of time intervals for *Cx. tarsalis* abundance. Variables for mosquito abundance and temperatures were centered at their mean values before fitting each model. The random intercept for each flock, θ_i, was shared by all chickens within the ith flock and served as an adjustment for landscape-scale, spatial variation in other confounders related to *Cx. tarsalis* abundance, and the potential for WEEV transmission.

RESULTS

WEEV activity, 1992–2000. WEEV activity, including virus-positive mosquito pools, sentinel chicken seroconversions, and a few cases of equine encephalomyelitis, occurred sporadically throughout the study period.[20,21] The years with the most intense activity were 1993, 1996, and 1997 in the Central Valley and 1992, 1995, and 1998 in the Coachella Valley (Table 1). The only year without WEEV seroconversions in chickens among those studied was 2000; 1999 had a single seroconversion. Most (33 of 41) flocks had seroconversions, and there was wide variation among flocks in the proportion of chickens that seroconverted. Seroconversions typically occurred earlier in the Coachella Valley than in the Central Valley (Figure 2), which might have been caused by higher temperatures and/or the earlier peak and subsequent decline in *Cx. tarsalis* abundance during the hot, dry summers.[38]

Temporal connections. Parameter estimates and odds ratios from the final regression model are presented in Table 2. The coefficient, γ, included to estimate the carryover of anomalies from each half-month to the next within a transmission season,

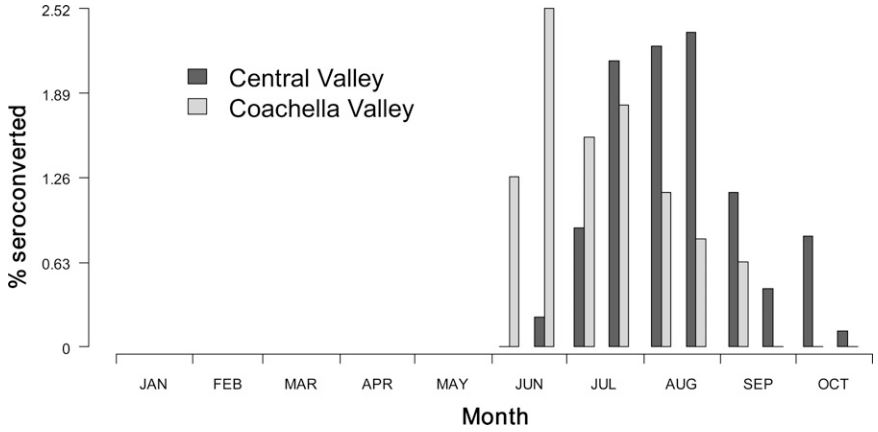

FIGURE 2. Percentages of chickens that seroconverted for western equine encephalomyelitis virus (WEEV) over the entire 1992–2000 study period for each half-month, Jun–Oct, in the Central and Coachella Valleys.

TABLE 2
Estimated regression coefficients with 95% credible intervals (CIs) and pertinent odds ratios from the final model

Coefficient	Mean (95% CI)	Odds ratio (95% CI)
Intercept	–8.015 (–8.915, –7.299)	
T_{MIN} 8–21d lag	0.201 (0.092, 0.314)	1.224 (1.096, 1.369)†
T_{MAX} 36–49d lag	0.105 (0.026, 0.186)	1.112 (1.026, 1.204)†
T_{MAX} 36–49d lag * Valley‡	–0.408 (–0.614, –0.219)	0.742 (0.614, 0.884)†
Cx. tarsalis ♀ 29–42d lag	0.653 (0.490, 0.823)	1.575 (1.404, 1.769)§
$\sigma_{among\text{-}flocks}$	0.748 (0.110, 1.465)	
γ	0.732 (0.639, 0.814)	

† Odds ratio (OR) based on a 1°C increase in temperature.
‡ Valley is a binary variable equal to 1 if Coachella Valley and 0 if Central Valley.
§ OR based on a doubling of relative *Culex tarsalis* female abundance.

was credibly positive and < 1.0, meaning that a probability of seroconversion above or below the mean in a particular half-month led to a similar departure during the following half-month, although the anomaly tended to slowly return toward the mean. Other autoregressive terms for temporal connections between years were evaluated (i.e., random effects connecting the final half-month of each season to the first half-month of the following season and fixed effects for the number or presence of WEEV seroconversions in the same flock the previous year), but none were credible (i.e., 95% CIs overlapped zero).

Temperatures. Temperatures within sliding 14-d intervals before each bleed date had much stronger associations with seroconversion risk in sentinel chickens than monthly temperatures for Jan–May, which did not have credible parameter estimates for any month or temperature variable. Based on DIC values, temperatures within windows of 8–21, 29–42, and 36–49 d before chicken sampling dates had the strongest associations with seroconversion risk. The interval 8–21 d before each chicken sampling date resulted in the best model fits for temperatures (i.e., lowest DIC values), and for that time window, minimum temperatures were best among the three variables considered. Among other intervals, maximum temperatures at 29–42 and 36–49 d lags provided the next best model fits, and their respective addition to models including $T_{MIN,8–21d}$ further improved the model fits (ΔDIC = –1.97 and –2.43, respectively). Because of the lower DIC value, $T_{MIN,8–21d}$ and $T_{MAX,36–49d}$ were retained as the temperature predictors in the final model.

Parameter estimates from the final model indicated that both $T_{MIN,8–21d}$ and $T_{MAX,36–49d}$ were positively associated with the odds of seroconversion (Table 2), with the association of $T_{MIN,8–21d}$ being about twice as strong as that of $T_{MAX,36–49d}$. Odds ratios indicated that an increase of 1°C in minimum temperatures 8–21 d before the sampling date led to an increase of 22.4% in the odds of seroconversion for WEEV. A 1°C increase in maximum temperature 36–49 d before the sampling date resulted in an 11.2% increase in seroconversion odds in the Central Valley.

The Coachella Valley had higher summer temperatures than the Central Valley, so we considered fitting a separate term for temperature variables within each valley to allow for different responses. Minimum temperatures at a 8–21 d lag had the same association in both valleys, but allowing for a different seroconversion response to maximum temperatures at a 36–49 d lag (Table 2) greatly improved the model fit (ΔDIC = –12.39). Odds ratios from the final model indicated that the 1°C increase in T_{MAX} that increased the odds of seroconversion in the Central Valley actually reduced the odds in the Coachella Valley by approximately 26%.

***Cx. tarsalis* abundance.** The association between relative *Cx. tarsalis* abundance and WEEV seroconversions was examined after adding the temperature variables described previously to the model. *Culex tarsalis* collections generally began in Apr of each year, so *Cx. tarsalis* per month for Apr, May, and Jun were considered individually for inclusion in the model. However, as for temperature variables, *Cx. tarsalis* abundance for staggered 14-d intervals before each sampling date resulted in much better model fits than the fixed monthly abundance values (DIC for the best lag interval = 29 lower than the best monthly predictor). Models were fitted for log-transformed *Cx. tarsalis* counts at each time interval and DIC values and parameter estimates were compared to look for trends and identify the most predictive time window(s). A pattern was evident in both DIC values and odds ratios (Figure 3), and *Cx. tarsalis* abundance at a lag of 29–42 d before the chicken sampling date clearly provided the best model fit. Adding an interaction term to estimate *Cx. tarsalis* parameters separately for the Central and Coachella Valleys did not improve the model's fit and the difference between the parameters for the two valleys was not credible. After adding the 29–42 d lagged term to the model, other non-overlapping lagged *Cx. tarsalis* counts were added, but again none improved the fit of the model, probably due in part to the high correlations between the *Cx. tarsalis*

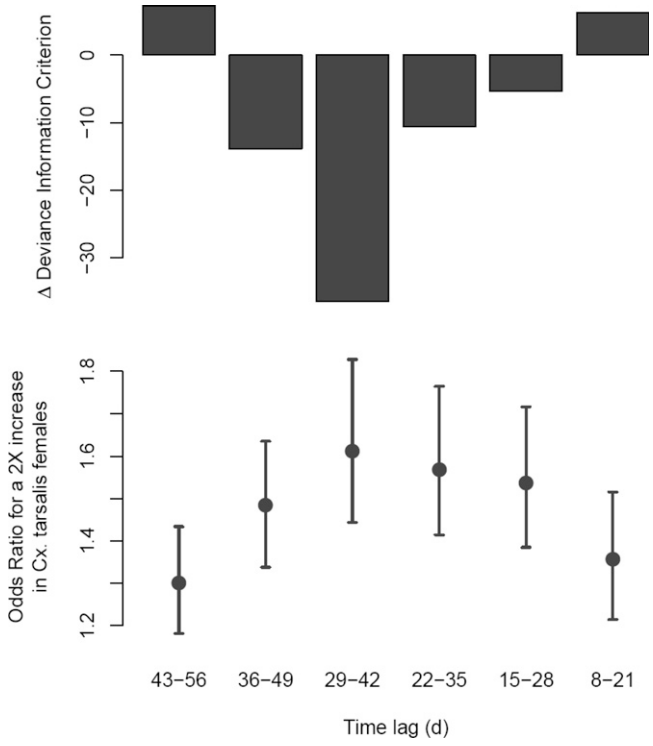

FIGURE 3. Changes in deviance information criterion (DIC) values on addition of the lagged *Culex tarsalis* predictor (upper panel) shown with odds ratios (ORs) with 95% confidence intervals (CIs) for western equine encephalomyelitis virus (WEEV) seroconversion for a 2-fold increase in relative *Cx. tarsalis* abundance (lower panel). Each DIC-OR pair represents a model fitted for the specified 2-week (14-d) interval before the bleed date for sentinel chickens.

lag periods (r = 0.72–0.84, $P < 0.0001$). A quadratic term was added to the linear term for relative *Cx. tarsalis* abundance at each time lag to account for the possibility suggested by Olson and others[9] that transmission would be reduced at the highest abundance levels, but none of the parameter estimates for squared terms were credible, indicating that enzootic transmission probabilities did not decline at the upper end of the abundance range observed in this study. Maximum temperatures at a 36–49 d lag were negatively correlated with *Cx. tarsalis* adult abundance one week later (i.e., during the 29–42 d lag) in the Coachella Valley (r = –0.29, $P < 0.0001$), but not in the Central Valley (r = 0.02, P = 0.36). The combination of high *Cx. tarsalis* abundance and extreme temperatures increased the probabilities of seroconversion in sentinel chickens (Figure 4), and the most pronounced increases in risk were at the upper limits (> 75th percentile) of the observed ranges for each variable, except in the Coachella Valley, where risk related to maximum temperatures was highest at the lower end of the range.

DISCUSSION

The transmission of WEEV to sentinel chickens clearly was associated with increased abundance of *Cx. tarsalis* after

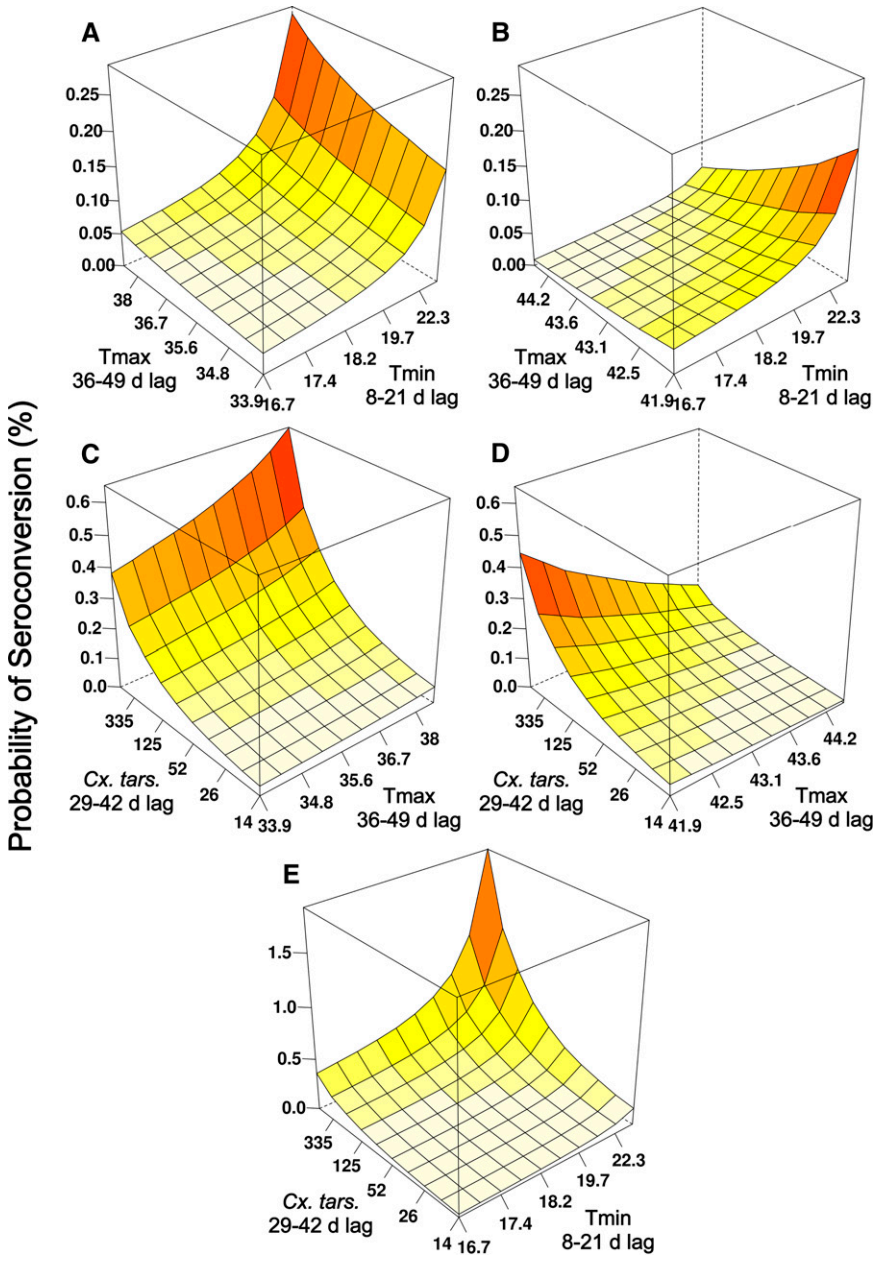

FIGURE 4. Predicted probabilities of biweekly seroconversion (height of surfaces) based on daily minimum temperatures (8–21 d lag), daily maximum temperatures (36–49 d lag), and *Culex tarsalis* ♀/2 weeks (29–42 d lag). Because associations of seroconversion probabilities with maximum temperatures differed between the valleys, associations are shown for the Central Valley (**A** and **C**), Coachella Valley (**B** and **D**), and both valleys (**E**). Grid lines represent the 50th–95th percentiles for each predictor. *Z*-axis scales are consistent within rows for comparisons between valleys. This figure appears in color at www.ajtmh.org.

adjustment for other confounders. For WEEV transmission risk to reach the highest levels, it seems that both antecedent vector abundance and temperatures must be at extreme levels, and neither abundance nor temperature alone was sufficient to dramatically increase risk.

An earlier study without adjustments for temperatures or other confounders[9] found that the incidence of human cases and seroconversions in sentinel chickens increased over the range from low to moderate seasonal *Cx. tarsalis* abundance, but was reduced during seasons with highest abundance. We found no evidence for a reduction in enzootic transmission at the upper end of the range of *Cx. tarsalis* abundance. The finer temporal scale of our study makes it difficult to compare directly with earlier results based on agency-level seasonal abundance indices, but it seems likely that the highest abundance levels recorded during Olson's 1953–1973 study period were not reached during our 1992–2000 study. Since the end of the Olson study period, the numbers of *Cx. tarsalis* collected in NJLTs have declined in many parts of California, attributed in part to actual abundance declines caused by urbanization of once-rural habitats and improved agricultural irrigation practices,[39] but also to declines in NJLT sensitivity caused by competing light from urban sources.[40,41]

Vector control remains the primary means for suppressing vector-borne pathogen transmission, and identification of a target vector abundance threshold below which transmission ceases is a desirable goal. Such thresholds are spatially and temporally dynamic, depending on the age structure of the vector population and immunity in the host population. Reeves[7,42] reported in Kern County, California that WEEV transmission ceased when seasonal NJLT indices were ≤ 1 *Cx. tarsalis*/trap-night and enzootic transmission persisted without human cases at seasonal indices from 1 to 10/trap-night. This study had finer spatial and temporal resolution and we did not directly estimate thresholds, but our results were qualitatively similar, with seroconversion probabilities asymptotically approaching zero following *Cx. tarsalis* female counts ≤ 1/trap-night (i.e., 14 females/2 trap-wks) and increasing rapidly for counts > 10/trap-night (140 females/2 trap-wks) (Figure 4). If such thresholds exist, they are likely to vary over space and time, because of differences in trap sensitivity and environmental factors, so additional study is needed to identify control targets at a finer spatial scale.

Summer temperatures in the Coachella Valley frequently exceed 40°C, and the reduction in transmission risk in the Coachella Valley at higher maximum temperatures may be caused by decreased adult survival so that females have a very short infective life span,[43,44] modulation of WEEV infection within *Cx. tarsalis*,[45] or a reduction in vector competence.[46] Increased larval mortality is associated with higher water temperatures,[44] and estimates of larval mortality from laboratory studies are likely to be conservative because temperature ranges selected for study are typically based on daily mean air temperatures. In natural shallow-water bodies, water temperatures exceed those of air for much of the day, by as much as 5°C.[47] Increases in larval or adult mortality at high maximum temperatures seemed plausible in the current study based on the negative correlation between maximum temperatures 36–49 d before chicken sampling and *Cx. tarsalis* adult abundance one week later in the Coachella Valley, but not in the Central Valley.

Mosquito abundance alone is not sufficient to quantify the entomological component of transmission risk for WEEV. Generally, parity is a prerequisite for transmission, with the probability of infection and transmission increasing with each gonotrophic cycle. As a result, older females pose a greater risk for transmission and the age structure of the population is an important determinant of transmission risk.[10] We could not assess age structure in our study, but it seems reasonable to expect that a large cohort of adult mosquitoes emerging at a particular time would carry forward to result in larger numbers of older females later in the season as the cohort ages. Other biological or behavioral factors of the population (e.g., autogeny and host-seeking patterns) also influence transmission risk, and these factors have not been quantified at the spatial and temporal scale of this study.

Sentinel chickens are cost-effective surrogates[48] for estimating enzootic WEEV transmission to wild avian amplifying hosts[49] and complement estimates of vector abundance and infection as part of comprehensive arbovirus surveillance programs. Serological monitoring of chickens remains the most consistent and comparable method for estimating WEEV transmission in California. Mosquito infection rates provide an indication that the virus is present, but the location at which the mosquito was infected cannot be pinpointed, except within the limits of its flight range. Furthermore, most infected mosquitoes are not capable of transmitting WEEV for any of several reasons, including mortality before completion of the extrinsic incubation period or infection and dissemination barriers within the vector.[50] Therefore, measuring infection rates does not necessarily provide an indication of transmission risk.[51] Wild birds are sometimes monitored for evidence of previous viral infection based on serologic testing. However, unless prior samples are available from the same bird or the bird is young enough to restrict the window of possible infection dates, the location and approximate date of infection usually is not known.

A limitation of serologic testing to detect WEEV transmission to chickens is that the rise in antibodies does not occur immediately upon infection. There is a delay of at least 8 d before antibodies reach diagnostic levels,[30] and a potential additional delay caused by weekly or biweekly sampling schedules. The date on which an antibody-positive blood sample was obtained from a sentinel chicken is not likely to be the exact date of seroconversion because the seroconversion could have occurred at any time after the previous bleeding date and before the one when antibodies were detected. To reduce the imprecision related to sampling intervals, California vector control agencies changed from monthly to biweekly sampling of sentinel chickens beginning in 1992. Therefore, during the period of our study, assuming a biweekly (14-d) bleeding schedule, the transmission event would have taken place 8–22 d before the date when the positive blood sample was obtained.

Surprisingly, temporal terms measuring between-year associations of WEEV seroconversion probabilities were not credible. Genetic evidence indicated that individual strains of WEEV have persisted in California over multiple seasons,[23] and a preliminary study at a coarser spatial scale[52] found that the odds of having ≥ 1 seroconversion within an agency tripled if at least one seroconversion had been detected the previous year. Taken together, these results suggested that the presence or absence of WEEV within a broad area tends to persist from year to year, but that the intensity of WEEV transmission at a specific site varies substantially from year to year.

The plausible timing of the associations and adjustments for confounders, including temperatures and flock-to-flock

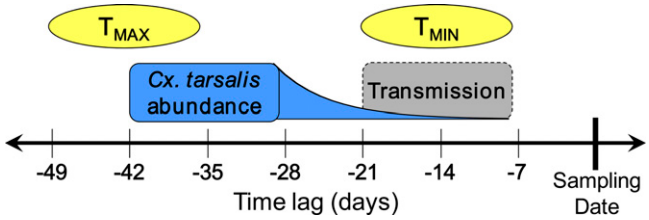

FIGURE 5. Schematic showing the *Culex tarsalis* abundance and temperature predictors from the final model, positioned at their respective critical time lags. Survival of *Cx. tarsalis* (80%/day) is depicted by the decay curve through the end of the transmission window for detection on the sampling date. This figure appears in color at www.ajtmh.org.

variation in transmission caused by unobserved spatial factors, lead us to believe that this abundance-transmission relationship is causal. The critical *Cx. tarsalis* abundance lag identified at 29–42 d preceding the sentinel chicken bleeding date is consistent with known transmission dynamics. The realistic scenario based on our final model (Figure 5) agrees well with the documented WEEV enzootic transmission in the Sacramento Valley during 1993.[11] If higher maximum temperatures during the 36–49 d lag window increase viral replication rates and shorten the extrinsic incubation period enough to enable already-infected adult *Cx. tarsalis* females to become infectious, these females could transmit WEEV to avian hosts within a few days. Competent avian hosts then would develop infective, circulating viremias over ~1–3 d,[53] just in time to infect host-seeking females peaking in abundance during the 29–42 d window. Assuming a realistic survivorship of adult *Cx. tarsalis* females of 80% per day,[10,54] some of these females would survive to infect chickens during the 8–21 d transmission window for the seroconversion to be detected at the following chicken sampling date. The combination of large numbers of older female *Cx. tarsalis* and increased minimum temperatures to reduce the extrinsic incubation period during the 8–21 d window would result in the increased probabilities of transmission. In the hotter Coachella Valley, a similar causal model would apply, with the exception that cooler maximum temperatures 36–49 d before chicken sampling would initiate the sequence of events leading to intensified transmission.

Decision makers in public health need adequate lead-time to guide vector control efforts to interrupt transmission before it reaches epidemic levels. The critical vector abundance window identified in this study 4–6 wks before the chicken sampling date highlights the need for a reduction of vector population densities before the detection of transmission to sentinel chickens. Temperatures before sampling dates also were associated with WEEV transmission, and the probability of seroconversion was highest when both temperatures and vector abundance were near the extreme ends of their observed ranges. Forecasting models incorporating vector abundance and temperatures may provide additional lead-time for public-health interventions.

Received June 13, 2009. Accepted for publication February 26, 2010.

Acknowledgments: We thank the local vector control districts listed in Table 1 for generously providing the mosquito collection records and maintaining the sentinel chicken flocks used in this study. We thank Andrew Michaelis, California State University, Monterey Bay and NASA Ames Research Center, for maintenance of the temperature data used in this study. We also thank the following individuals for helpful input during this research: Jeff Gill, Center for Applied Statistics, Washington University in St. Louis; Tim Carpenter, Center for Animal Disease Modeling and Surveillance, University of California, Davis; Susan Ustin, Center for Spatial Technologies and Remote Sensing, University of California, Davis; and Thomas Scott, Department of Entomology, University of California, Davis. We also benefited from discussions with fellow members of the Research and Policy for Infectious Disease Dynamics (RAPIDD) program of the Science and Technology Directorate, U.S. Department of Homeland Security, and the Fogarty International Center, National Institutes of Health.

Financial support: This work was funded by NASA Earth-Sun Science Applied Sciences Program Research Opportunities in Space and Earth Science, Decision Support through Earth-Sun Science Research Results grant RM08-6044 for NNA06CN02A and NOAA Office of Global Programs, Climate Variability and Human Health grant 00-543.

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